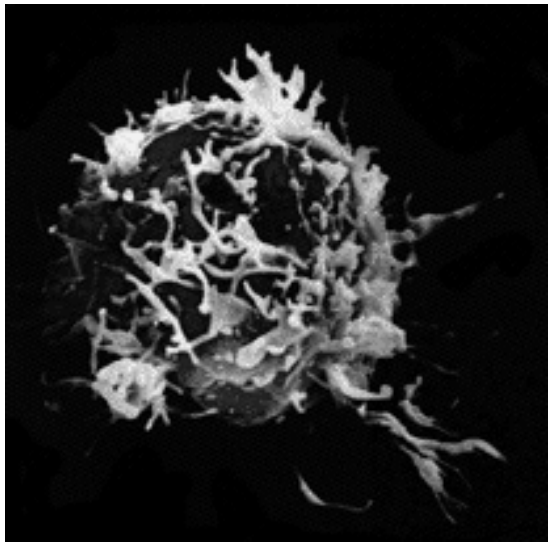
The Los Alamos Immuno-Lymphocyte Proliferation Test

Babetta L. Marrone, Ph.D.
Group Leader, Cytometry Group
Life Sciences Division
Los Alamos National Laboratory
blm@lanl.gov

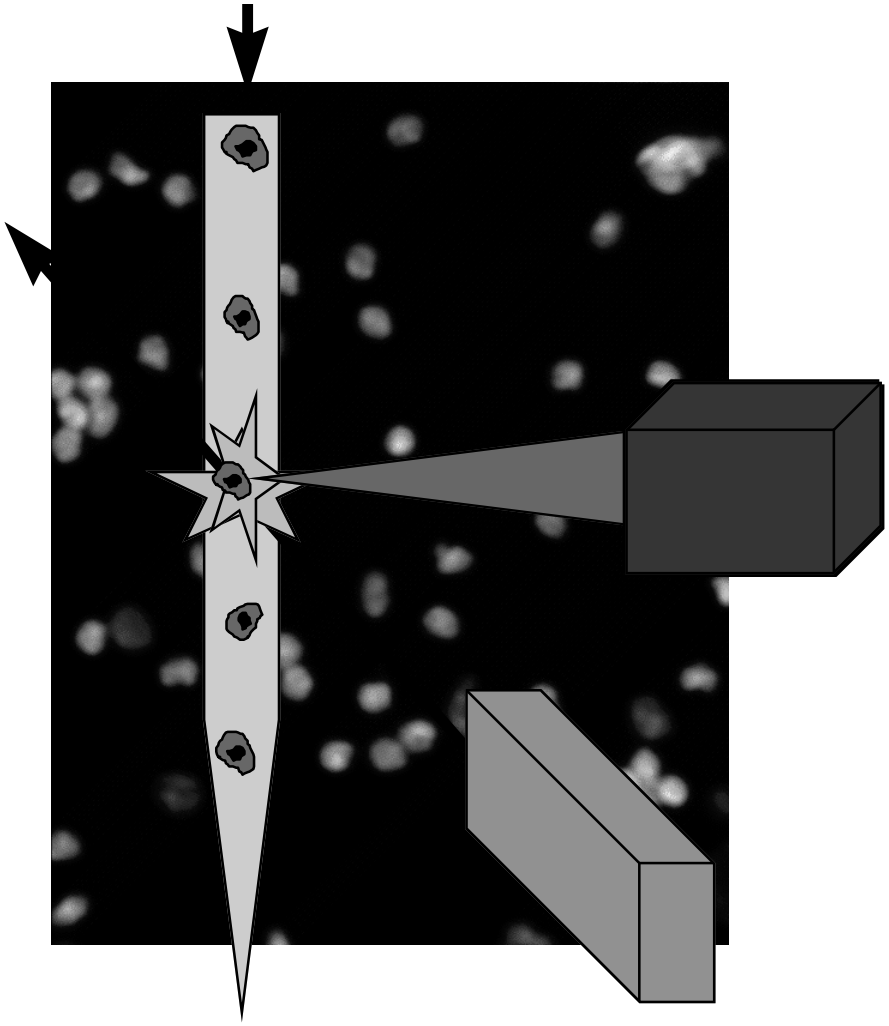
LANL LPT Program

- **Development of the Immuno-LPT and Application to Medical Surveillance of LANL Beryllium Workers**
  - **» >300 workers**
  - **» Informed consent(s) in place**
  - **» Testing has begun**
  - **» 100 workers tested so far**
  - **» Split samples sent to NJC**
  - **» NJC CBD samples sent to LANL for test validation and research**



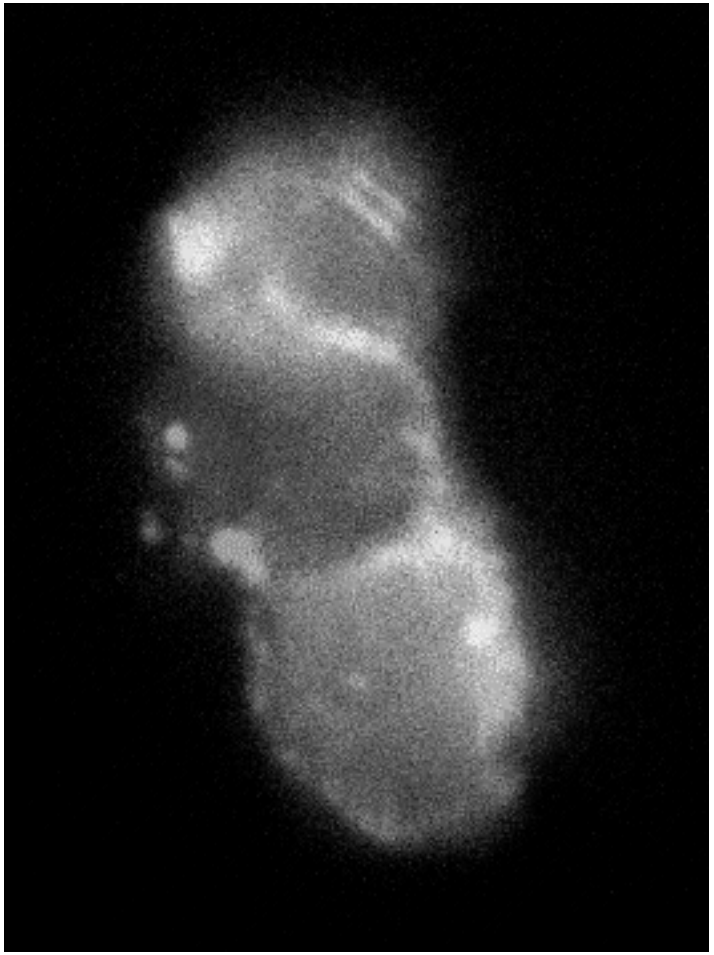
Goal and Approach

- **Develop LPT with greater predictive value for CBD**
- **Base LPT on Flow Cytometry**
  - **More sensitive**
    - **single cell analysis**
  - **More specific**
    - **lymphocyte subset analysis**
  - **More informative**
    - **several measures obtained from the same sample**

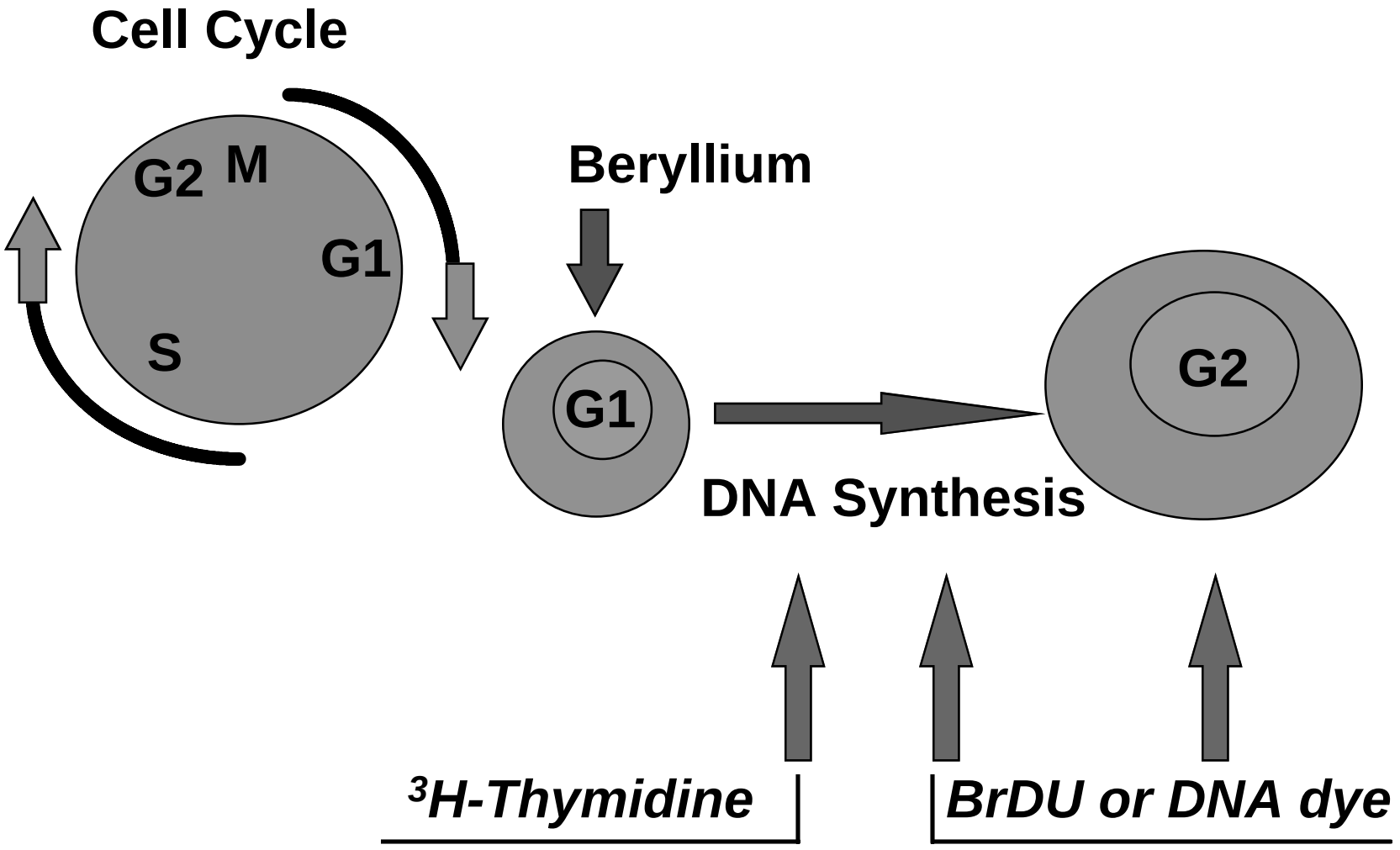


The Immuno-LPT

- Rationale: CBD is an immune disease, involving T helper cells
- Measure CD3, CD4, CD8, and proliferation in same sample using Multiparameter Flow Cytometry
- Express % proliferating cells in terms of cell subset



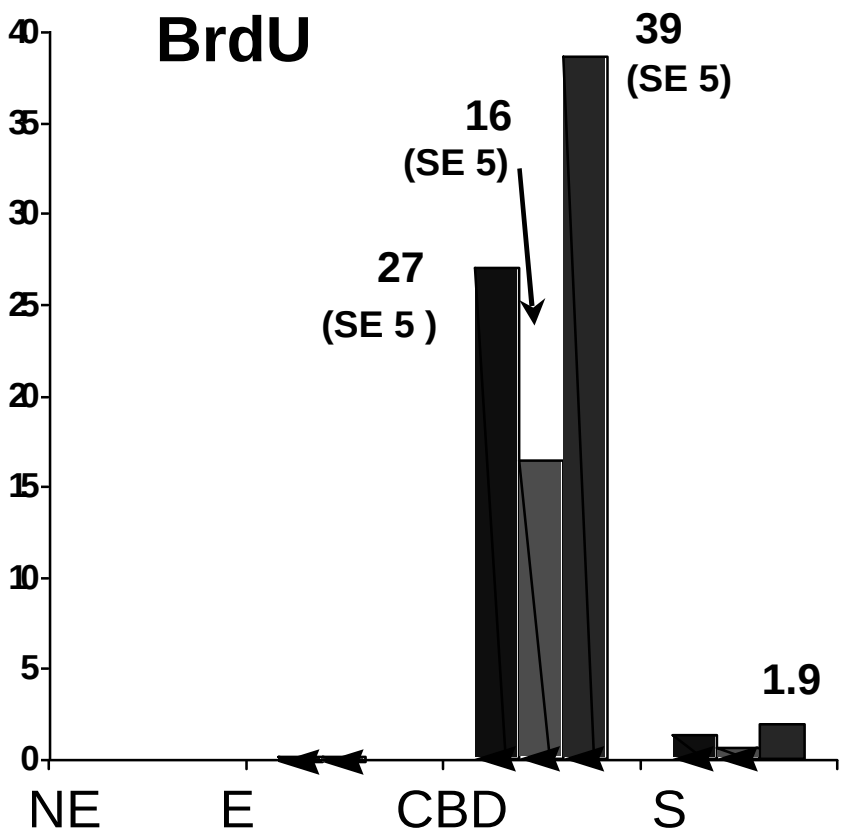
The LPT Measures Cell Proliferation in Response to Beryllium

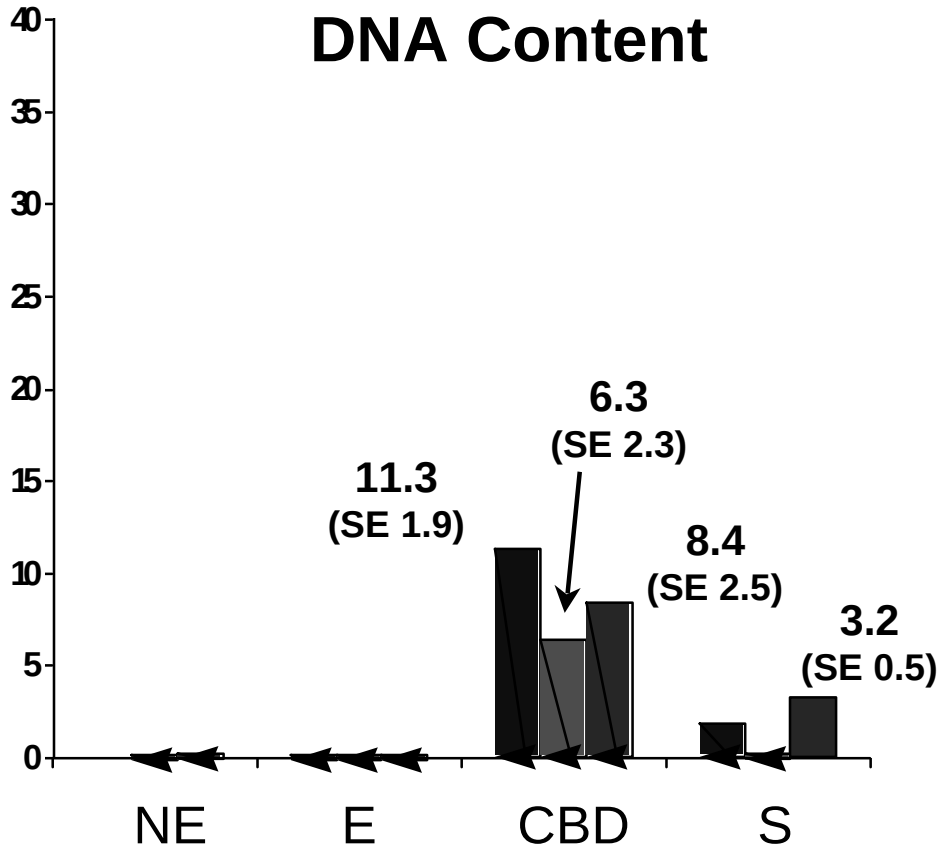


Two Immuno-LPTs Have Been Developed

- **Screening Immuno-LPT**
  - **BrDU incorporation following Be challenge in CD4 and CD8 subsets**
  - ***Purpose*: Analogous to Thymidine incorporation assay**
- **Confirmatory Immuno-LPT**
  - **DNA content assay following Be challenge in CD4 and CD8 subsets**
  - ***Purpose*: Confirm proliferation in BrDU-positives**

Average % Replication after 7d Culture with 10 µM Be and Human Serum (Difference from Media Control)





All lymphocytes
CD4 lymphocytes (Helper)
CD8 lymphocytes (Suppressor/Cytotoxic)

NE - Not Exposed
E - Exposed
CBD - Chronic Beryllium Disease
S - Sensitized

“Other Sensitives”

#	Exp.*	BrDU CD4	BrDU CD8	DNA CD4	DNA CD8
1)	F	0.2	0	0.4	2.5
2)	F/C	0.1	0	0.2	2.2
3)	F	1.7	2.2	0.2	4.2
4)	F/C	0	0.9	0	4.4
5)	C	0.2	0.9	0.8	4.3
6)	NE	0	0	0	5.0

Assay results from 10 or 100μM Be and human serum

Exp*:
F= former, C=current, NE= nonexposed

Conclusions

- 2 Immuno-LPTs based on flow cytometry have been developed
  - screening
  - confirmatory
- Lack of a confirmed CD4 response may distinguish CBD from sensitive
  - NEED MORE DATA
- Plasma (autologous) maximizes CD4 cell response
- Serum (non-autologous) maximizes CD8 cell response